CECE Junior 2025

November 11 - 12, 2025
Institute of Analytical Chemistry of the CAS
Brno, Czech Republic

Program and Abstract Book

"… bringing people and ideas together …"

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Foreword

CECE Junior 2025 is dedicated to Ph.D. students, postdocs, and early-career scientists. It offers a friendly platform to present research in chemistry, biotechnology, and related disciplines, emphasizing networking and scientific development for junior researchers. A poster session complements a two-day program of oral presentations.

Acknowledgement

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Organizing committee

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CECE Junior 2025 – Scientific Program

Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

11.11.2025		
8:00-9:00	Registration	
9:00-9:15	Opening *František Foret, Roman Řemínek*	
9:15-10:00	PL1	Capillary electrophoresis - unnecessary due to AI? *Hermann Wätzig*
10:00-10:20	O1	Key factors influencing the efficiency and reproducibility of double-chained cationic surfactant coatings for protein separation in capillary electrophoresis *Chirapha Prakobdi*
10:20-10:40	O2	Taylor dispersion analysis of liposomes and the effect of carrier ampholytes on their integrity *Pavlína Dadajová*
10:40-11:10	Coffee break	
11:10-11:30	O3	Droplet microfluidics: A high-throughput platform for enzyme kinetics, inhibition screening, and therapeutic agent development *Lukáš Jordán*
11:30-11:50	O4	Development of micro-blot array diagnostic kit with nanoparticle-conjugated antigens for serological diagnosis of tropical diseases *David Pokorný*
11:50-12:10	O5	Label-free micro- and nanoparticle quantification via evaporated volume analysis (EVA) *Julie Weisová*
12:10-12:30	O6	Optical microscopy and evaporation-based analysis for quantification of nanoscale entities *Kateřina Uhrová*
12:30-13:00	Lunch break	
13:00-14:30	Poster session + Lab visit - Institute of Analytical Chemistry	
14:30-14:50	O7	HILIC-MS/MS for precise glutathione metabolism profiling *Miroslav Kubát*
14:50-15:10	O8	Simultaneous mapping of peptides and released N-glycans by HILIC-FLD-MS for quality control of protein biopharmaceuticals *Mykyta R. Starovoit*
15:10-15:30	O9	Patient-friendly LC–MS therapeutic drug monitoring in alternative matrices *Viktória Ďurčová*
15:30-15:50	O10	Modulation of high-pH mobile phase incompatible with the 2nd dimension column for SAX-RPLC of peptides *Derya Demir*
15:50-16:10	O11	Reaction kinetics analysis of fungal hyaluronidase TsHr *Jana Jílková*

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12.11.2025		
8:00-9:00	Registration	
9:00-9:45	PL2	Getting the balance right: Reflections on a career bridging science and business *Marek Minárik*
9:45-10:05	O12	Early type 2 diabetes screening by combining high-resolution capillary electrophoresis separation and machine learning supported data evaluation *Rebeka Török*
10:05-10:25	O13	Analysis of brain tissue N-glycosylation using capillary electrophoresis *Beatrix Kiss*
10:25-10:55	Coffee break	
10:55-11:15	O14	Data-driven gradient optimization in LC-MS/MS: From semi-experimental framework to applications in food phenolic analysis *Michal Kašpar*
11:15-11:35	O15	Fully automated sample preparation in-syringe online coupled to HPLC for vitamins analysis in vegan milk *Tar Tar Moe Htet*
11:35-11:55	O16	Automated analysis of proteins in dried plasma spot samples *Helena Hrušková*
11:55-12:15	O17	Nanofiber-based microextraction for environmental analysis *Ewelina Czyz*
12:15-12:35	O18	An automated platform for the monitoring of microfluidic immobilized enzyme reactors *Sanjay Lama*
12:35-13:30	Lunch break	
13:30-13:50	O19	Novel multipurpose matrix for a high resolution MALDI MS and multimodal imaging *Michal Javorek*
13:50-14:10	O20	Impedance-based detection of biofilm formation of *Staphylococcus aureus* and *Staphylococcus epidermidis* *Bengisu Yöney*
14:10-14:30	O21	Bioconjugates of upconversion nanoparticles for immunochemical detection of tumor biomarkers *Pavel Špaček*
14:30-14:50	O22	Physics for sensitivity, chemistry for selectivity: Planar spark and Ag/FexOy colloid substrates for SERS *Vít Pavelka*
14:50-15:10	O23	Development of microfluidic capillary electrophoresis with surface-enhanced Raman scattering detection (CE–SERS) *Lucie Březinová*
15:10-15:30	O24	Deuterated water amplified fluorescence detection in capillary electrophoretic separation of DNA *Jan Badin*
15:30-15:50	O25	A decade of capillary electrophoresis–mass spectrometry in precision medicine: A global bibliometric study (2014-2024) *Letícia Gaiola*
15:50-15:55	Closing *František Foret, Roman Řemínek*	

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Poster presentations	
P1	Absolute quantification of nanoparticles and nanoplastics for their chemical characterization *Hana Brožková*
P2	Fluorescein-based markers of isoelectric point as a tool tracking of pH gradient in highly sensitive isoelectric focusing analysis with laser-induced fluorescence detection *Pavlína Dadajová*
P3	Characterization of linear and branched cuticular hydrocarbons in *Blaptica dubia* using GC/Q-TOF and molecular sieve separation *Kateřina Hrabáková*
P4	Chromatographic techniques for the analysis of historical pharmaceuticals of plant origin *Tomáš Lener*
P5	Bringing liquid chromatography out of the lab: A compact UV-Vis detector for portable systems *Zuzana Maďarová*
P6	Co-cultivation of *Hypericum*-borne endophytes unlocked anthraquinone biosynthesis *Martina Matoušková*
P7	Acoustic alignment of bacteria toward piezoelectric biopolymer devices *Jakub Vejrosta*
P8	Optimization of electrospray ionization for MS/MS sequencing of Substance P *Adéla Veselá*

Note: Please prepare your poster in A0 portrait format.

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PL - plenary lecture

O – oral presentation

P – poster presentation

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PL1

Capillary electrophoresis - unnecessary due to AI?

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Summary

In recent years, there have been numerous advances and new developments in the field of capillary electrophoresis (CE). The pros and cons of the various instruments are becoming increasingly well understood, and the rules for method development have been clarified, particularly with regard to larger molecules, including biopharmaceuticals. CE-MS has evolved significantly and is now increasingly becoming a routine option for quality control. Last but not least, affinity CE assays complement the range of instruments available for binding assays.

Fundamentals form the basis of every science. For CE, this foundation was largely laid by Czech scientists. This has resulted in numerous publications, and to highlight one in particular: the CEval software has established itself as the standard tool worldwide.

“Artificial intelligence” will not replace clever algorithmic solutions. Algorithmic solutions will remain important, but in some cases will be improved by AI. AI holds many promises – and as always, promises are sometimes kept.

Peak integration in CE remains a challenge. There are numerous ideas for improving peak integration, ranging from simple algorithms for improving the signal-to-noise ratio to more complex AI solutions. However, none of these ideas can prove that they are genuine improvements, as they cannot be compared with each other or with previous algorithms using the same reference data sets. Therefore, data sets were collected, standardized, and then integrated by teams of experts. This consolidated data will soon be available to the scientific community.

So, to answer the question in the title: NO! However, when used sensibly, AI can significantly improve science in many areas, including CE.

PL2

Getting the balance right: Reflections on a career bridging science and business

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Summary

This lecture will present a personal perspective on building a career that integrates scientific rigor with entrepreneurial vision. Drawing upon experiences from both academic and industrial environments, I will discuss the importance of a strong educational foundation and the ability to connect fundamental research with real-world challenges.

A strong influence on my outlook was gifted to me from my father, a distinguished scientist at the Czechoslovak Academy of Sciences, who transitioned from a scientist working in an environment shaped by the communist era into a successful entrepreneur. My professional journey has been rooted in the field of separation science, beginning with fundamental research in physical chemistry focused on capillary electrophoresis (CE). Over the years, my field of interest evolved toward applied research and the development of CE instrumentation, particularly the use of capillary electrophoresis for DNA analysis. It subsequently extended into genetic and genomic testing — including DNA mutation analysis and DNA sequencing — and culminated in my current focus on cancer diagnostics, where molecular methods are applied to therapy monitoring and the early detection of disease recurrence.

Academic training and years at a U.S. university played a pivotal role in shaping my appreciation for methodological rigor, critical thinking, and the pursuit of excellence in research. Later, my transition into the biotechnology industry in Silicon Valley provided a unique opportunity to explore the interface between scientific innovation and technology-driven business. These experiences also offered insight into the values and ambitions often associated with the “American dream”. So when returning to Prague I tried to transfer not only the knowledge, but also the immense spirit of enterprise, a desire to learn new things, and, of course, a great deal of desire for success and recognition. Often accompanied by locally specific circumstances, I managed to achieve some of it, including a taste for fleeting media attention based on unique projects in the then-emerging field of recreational genetics. But from my point of view, the most satisfying fact would be the maintenance of a high level of scientific projects, the ability to produce quality publications and performing a high-regarded clinical service to cancer patients while maintaining a moderate success in the commercial arena.

There is no doubt all I owe a lot to interdisciplinarity - the ability to translate expertise from one domain to another — and on the lifelong challenge of balancing diverse interests while maintaining focus the important factors in life.

O1

Key factors influencing the efficiency and reproducibility of double-chained cationic surfactant coatings for protein separation in capillary electrophoresis

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Summary

Capillary electrophoresis (CE) of intact proteins remains a challenging yet highly valuable technique because of its potential to separate and quantify clinical biomarkers [1,2]. To make this approach more effective, CE methods need to deliver high separation efficiency, good reproducibility, and minimal protein adsorption on the capillary wall. Among the different strategies developed to address these issues, coatings based on double-chained surfactants have shown great promise in improving overall performance [3,4].

In this work, we investigated dioctadecyldimethylammonium bromide (DODAB), a double-chained cationic surfactant, as a coating material for CE. Our goal was to identify the main factors that influence how DODAB vesicles form and how the coating performs during protein separations. We systematically studied the effects of temperature and sonication during coating solution preparation, the composition of the coating buffer, and the inner diameter of the capillary.

The separation performance was evaluated by plotting the plate height (H) against the linear velocity (u) at different voltages to understand how the coating behaves under various conditions [5]. The DODAB coating provided a smooth and uniform surface, reflected by a low average slope of the H–u curve (~2×10^{-3} s, n = 3 capillaries) for five model basic proteins, lysozyme, carbonic anhydrase, ribonuclease A, β-lactoglobulin A, and myoglobin using a 40 cm total length and 25 µm inner diameter capillary. This coating enabled efficient separations, with lysozyme showing the best result at −12 kV, corresponding to ~600,000 plates/m, and excellent repeatability (RSD of migration time ~ 0.2 %).

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O2

Taylor dispersion analysis of liposomes and the effect of carrier ampholytes on their integrity

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Summary

Liposomes are spherical vesicles made of phospholipid bilayer widely used as biomimicking models and drug delivery vesicles. The isoelectric point (p*I*) of liposomes as an evaluation parameter has been measured only sporadically. In fact, liposomes have the potential to exhibit changes in the net surface charge depending on their composition and the pH of their enviroment [1]. Capillary isoelectric focusing (cIEF) can determine p*I* in a single measurement, but application of protein-centric cIEF methods to liposomal drugs and lipid nanoparticles often results in a sample aggregation or disruption [2]. Taylor dispersion analysis (TDA) is an absolute method for determination of average diffusion coefficient and subsequently hydrodynamic radius R_h. TDA can be applied for the size characterization of macromolecules and lipid nanoparticles [3,4] and is capable of detecting multiple vesicles populations of different R_h in one sample. This study demonstrates that TDA can be applied for size characterization of model liposomes, and monitoring of their stability during interaction with carrier ampholytes (CA), which are crucial for a cIEF separation. Concentration ranges of CAs, where aggregation does not occur, were determined. Comparisons of CA systems revealed that Pharmalytes 3-10 generated a more stable linear gradient but destabilize liposomes already at concentration 0.2 % w/w. Meanwhile, AESlyte SH 3-10 appear to be the less disruptive option towards liposomes, and the integrity of vesicles is preserved up to 0.5% w/w. Although, AESlytes create less linear pH gradient at given concentration during a cIEF separation. These findings represent a critical step toward the development of a robust and reliable cIEF method for lipid-based systems.

This research was co-funded by the European Union under the ATEBIO project (Advanced Techniques for Biomedical Diagnostics, Project ID CZ.02.01.01/00/23_020/0008535). This work was also supported by the institutional research plan [RVO:68081715].

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O3

Droplet microfluidics: A high-throughput platform for enzyme kinetics, inhibition screening, and therapeutic agent development

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Summary

Enzyme kinetics is foundational to modern biochemistry and drug discovery, providing critical insights into reaction mechanisms and guiding the rational development of therapeutic enzyme inhibitors[1]. Accurate determination of kinetic parameters (KM and Vlim) and inhibition constants (Ki) is essential for developing drugs that specifically target enzymes involved in metabolic pathways. However, conventional assay methods are often limited by high sample consumption, significant reagent costs and limited throughput, which can hinder large-scale screening efforts.

This work overcomes these limitations by using droplet microfluidics as a powerful, miniaturised platform. Droplet systems confine reactions within nanoliter-sized volumes, effectively turning each droplet into an isolated, highly efficient microreactor. This approach offers several transformative advantages, including massive parallelisation (i.e. high-throughput screening testing), extremely low reagent consumption and precise control over reaction conditions [2].

This presentation introduces a novel microfluidic methodology for the comprehensive analysis of enzyme kinetics and inhibitor dose-response screening directly within monodisperse droplets. The platform's efficacy was verified using the established model enzyme, E. coli β-galactosidase. The integration of rapid droplet generation, precisely controlled incubation, and high-speed fluorescence detection has yielded significant reductions in assay time and reagent consumption. Ultimately, the kinetic and inhibition constants derived from the microfluidic data were quantitatively equivalent to results from macroscopic bulk assays.

In conclusion, droplet microfluidics is a robust and efficient technology that significantly accelerates the study of enzyme function and the search for potent inhibitors. This powerful platform is set to become a core tool for developing next-generation therapeutic agents.

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O4

Development of micro-blot array diagnostic kit with nanoparticle-conjugated antigens for serological diagnosis of tropical diseases

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Summary

The growing incidence of tropical diseases such as dengue, chikungunya, or Zika – especially their recent emergence in Europe – highlights an urgent need for reliable and sensitive diagnostic tools. Micro-Blot Array (MBA) is an effective and promising method for antibody diagnostics with many advantages in comparison with other traditional methods (ELISA or Western Blot). This project aims to design and develop a new MBA diagnostic kit for the detection of mentioned diseases-specific antibodies in human serum or plasma and then finally launch it to the market.

The project follows a multi-phase development plan: comprehensive theoretical research on the target diseases and current diagnostic strategies, identification and optimization of kit components (antigens, buffers etc.), refinement of the production process, and detailed evaluation of the kit's analytical performance in preparation for IVDR certification. A key innovation in this work is the integration of nanoparticle-conjugated antigens, which makes the kit to be the first of the 3rd generation of MBA. We expect this to enhance assay sensitivity, stability, and other analytical properties.

The work on this project includes standard processes of the MBA production (micro dispensing antigens on NC membrane, assembling it to the MTPs and performing standardized detection with samples of plasma or serum). An essential parallel task is the development of an optimal protocol for antigen-nanoparticle conjugation. Continuous improvement efforts are also focused on increasing production robustness and efficiency. Currently, the project is in the component testing phase.

Expected outcomes include a fully functional, high-performance MBA kit ready for clinical validation and certification. In conclusion, this project seeks to establish a next-generation diagnostic tool for tropical diseases with improved performance and potential for global public health impact.

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O5

Label-free micro- and nanoparticle quantification via evaporated volume analysis (EVA)

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Summary

We recently introduced a novel absolute method for estimating nanoparticle concentration, known as evaporated volume analysis (EVA). In this approach, microvolumes of nanoparticle dispersions are deposited on a glass substrate, the solvent is evaporated, and the entire dried droplet area (several mm^2) is imaged by optical microscopy. Multiple micrographs are stitched together, and individual nanoparticles—typically several thousand per droplet—are recognized and counted using a convolutional neural network.

Originally, EVA was developed for photon-upconversion nanoparticles and implemented in epiphoton-upconversion microscopy mode. Here, we discuss adapting EVA for brightfield optical microscopy, enabling the analysis of micro- and nanoparticles without the need for intrinsic fluorescence or fluorescence labeling. Brightfield-EVA employs a customized neural network algorithm that allows direct, label-free detection and counting of light-absorbing and scattering nanoparticles. We investigate its applicability across nanomaterials of different sizes and compositions, including polystyrene, silica, and plasmonic nanoparticles. We envision that by combining absolute quantification, and simplicity, brightfield-EVA could offer a versatile and accessible approach to nanoparticle characterization in material sciences and bioanalytical chemistry.

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O6

Optical microscopy and evaporation-based analysis for quantification of nanoscale entities

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Summary

Quantification of nanoparticles and nanoscale biological entities, such as extracellular vesicles, viral particles, proteins, and other molecules, is a central challenge in analytical chemistry and the life sciences [1-2]. Here, we present an optical microscopy-based method termed evaporated volume analysis (EVA) that enables absolute quantification and imaging of individual nanoparticles in microliter-scale samples after solvent evaporation. EVA combines straightforward sample preparation with large-area imaging and automated particle counting, offering reproducible and accurate number concentrations. We validated the method on different types of nanoparticles, demonstrating broad applicability and excellent agreement with established reference techniques [3].

This methodology offers a simple, rapid, and quantitative platform suitable for applications in analytical chemistry, nanomedicine, diagnostics, and fundamental biological research. Overall, our work highlights the potential of optical microscopy as a practical tool for routine analysis of nanoscale entities, bridging the gap between qualitative imaging and quantitative particle counting.

The authors acknowledge grant 24-11183S from the Czech Science Foundation. The publication was produced with support for the long-term conceptual development of the research organizations from the Institute of Analytical Chemistry of the Czech Academy of Sciences (RVO: 68081715).

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O7

HILIC-MS/MS for precise glutathione metabolism profiling

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Summary

Hydrophilic interaction chromatography (HILIC) serves as a robust liquid chromatographic technique for separating polar, hydrophilic, or ionizable compounds that demonstrate inadequate retention in reversed-phase chromatography, encompassing biogenic analytes such as amino acids, peptides, sugars, oligosaccharides, nucleotides, oligonucleotides, polar metabolites, and pharmaceutical conjugates with glutathione, cysteine, or glucuronic acid. Its seamless integration with mass spectrometry (MS) stems from the ionizable properties of many polar compounds, which typically lack chromophores for effective UV detection but provide strong MS signals; commercially available HILIC stationary phases include bare silica, modified silica, zwitterionic variants, and hybrid or polymer-based materials offering enhanced chemical stability [1]. HILIC proves especially effective for targeted analysis of glutathione (GSH) metabolic and detoxification pathways, where GSH functions as the principal intracellular antioxidant, a marker of oxidative stress, and a biomarker for chronic diseases including diabetes mellitus, cystic fibrosis, osteoarthritis, renal and hepatic disorders, cardiovascular conditions, and neurodegeneration, thereby facilitating deeper insights into metabolism that could enhance therapeutic interventions for intoxications and symptom relief in chronic illnesses [2]. In this work, an HPLC-MS/MS method was developed employing a ZIC-HILIC column with fully porous particles on an Agilent 1290 Infinity II LC system coupled to an Agilent 6475 triple quadrupole MS in electrospray ionization (ESI) mode; optimization involved gradient elution in HILIC mode and multiple reaction monitoring (MRM) transitions in positive ion mode, with 0.05% (v/v) difluoroacetic acid identified as an optimal mobile phase additive to balance selectivity and sensitivity. Sample preparation was optimized using 5% sulfosalicylic acid, assessed for extraction recovery, matrix effects, acid-induced artifacts, and protein precipitation efficiency, revealing substantial matrix- and acid-related influences on detector response that prompted comprehensive validation through multiple standard additions. The resulting validated method enables selective and sensitive quantification of 21 GSH- and sulfur-related metabolites in cellular samples without derivatization, and its application to A549 lung cancer cells exposed to $CdCl_2$ and cisPt uncovered metabolic alterations, underscoring its potential in toxicological and oncological investigations.

The work was financially supported by the Czech Science Foundation, project No. 22-09556S.

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O8

Simultaneous mapping of peptides and released N-glycans by HILIC-FLD-MS for quality control of protein biopharmaceuticals

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Summary

Protein biopharmaceuticals, especially monoclonal antibodies, have revolutionized therapeutic regimes. However, their structural complexity presents analytical challenges. Modifications such as glycosylation and deamidation affect efficacy and immunogenicity, demanding precise monitoring of critical quality attributes (CQAs). Current multi-attribute methods, based on reversed-phase (RP) LC-MS of tryptic peptides, struggle with short hydrophilic peptides and show limited resolution of deamidated, isoAsp-containing, and glycosylated peptides. Glycosylation assessment often requires separate workflows with enzymatic release, fluorescent labeling, and HILIC-FLD analysis.

We propose a UHPLC method that combines the mapping of tryptic peptides with released, RapiFluor-MS-labeled N-glycans in a single HILIC-FLD-MS run, utilizing convenient sequential injection of peptide and glycan fractions. The approach separates peptides in the initial part of the chromatogram, followed by glycans in later retention windows, with fluorescence labeling enhancing glycan detection. Coupled with tandem mass spectrometry, this approach provides full protein sequence coverage and superior resolution of isomeric glycoforms, deamidated peptides, and isoAsp residues versus RPLC workflows. Analysis of released, labeled glycans further outperforms glycopeptide separation, both by RPLC and HILIC, in preserving terminal sialic acids that are prone to in-column cleavage at acidic pH and high column temperatures.

This integrated HILIC workflow could simplify biopharmaceutical quality control by enabling the simultaneous monitoring of multiple CQAs, which now require distinct methods, thereby improving efficiency, reliability, and the cost per sample.

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O9

Patient-friendly LC–MS therapeutic drug monitoring in alternative matrices

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Summary

Therapeutic Drug Monitoring (TDM) is crucial for optimizing antiseizure medication (ASM) therapy in epilepsy management. However, patients often show considerable variability in ASM concentration due to differing pharmacokinetics. Conventional TDM is based on venous blood sampling, which can be invasive and less practical in outpatient or home settings. This work presents a developed LC–MS method for simultaneous measurement of seven ASMs in three alternative matrices: saliva, dried saliva spots (DSS) and dried blood spots (DBS). These matrices offer clear advantages for clinical use: saliva sampling is completely non-invasive, DBS requires only a small finger prick, and both simplify sample handling, storage and transport.

Quantification of ASMs is reliably accomplished from 20 µL of saliva and 45 µL of capillary blood. Sample preparation employed protein precipitation with a methanol:acetonitrile:water mixture (7:2:1, v/v/v), efficiently recovering all analytes for all types of matrices. Calibration curves demonstrated linearity across relevant therapeutic ranges: 1–100 µg/mL for most drugs, 10–150 µg/mL for valproic acid in DBS, and 0.5–50 µg/mL in saliva and DSS. For the selected model compound, lamotrigine, analysis of twenty-seven parallel patient samples demonstrated a robust linear correlation between plasma concentrations and concentrations measured in both saliva (R=0.847) and DSS (R=0.839). Ten DBS samples showed R value of 0.88. These preliminary data confirmed feasibility of using these alternative matrices, and further quantitative studies are currently underway to comprehensively evaluate their correlation with established plasma concentrations.

This LC–MS approach provides a promising foundation for non-invasive TDM of ASMs and holds potential for more personalized and accessible patient monitoring.

This work was financially secured by Czech Health Research Council, project No. NU23-08-00229.

O10

Modulation of high-pH mobile phase incompatible with the 2nd dimension column for SAX-RPLC of peptides

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Summary

Two-dimensional liquid chromatography is a powerful analytical approach for thorough separation of complex samples, utilizing different chromatographic modes [1]. However, the mobile phase pH incompatibility arising from different retention mechanisms between the modes can lead to retention time shifts, reduce the reproducibility and reliability of the analysis, and, in the worst scenario, compromise column longevity [2]. To address this issue, high-volume sampling loops or make-up pumps can be used, but it remains crucial to ensure that the dissimilar pH does not negatively affect either the subsequent column or the integrity of the analysis [3]. During the development of our 2D-SAX-RPLC system employing a column with an inner diameter of 1.5 mm, we were particularly attentive to this problem. To evaluate whether implementing a simple inline mixing capillary can effectively dilute the eluent from the 1st dimension SAX and reduce its pH, we employed several measurement strategies to monitor the pH both after the mixing capillary and following transfer to the 2nd dimension via the switching valve. pH variations were monitored using methyl red as an indicator, where the observed color change reflected the corresponding pH shift. Our findings demonstrate a simple yet effective strategy to control inter-dimensional pH transitions, ensuring reliable and reproducible performance of the 2D-LC system.

This study was supported by the Project of the Czech Science Foundation (GAČR No. 22-21620S), the SVV project No. 260782, and the ATEBIO project (Advanced Techniques for Biomedical Diagnostics, Reg. No. CZ.02.01.01/00/23_020/0008535) co-funded by European Union.

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O11

Reaction kinetics analysis of fungal hyaluronidase TsHr

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Summary

Fungal hyaluronidases have not received much attention among hyaluronan degrading enzymes. These enzymes are structurally distinct from mammalian and venom hyaluronidases, although their reaction products are identical. Their non-animal origin and possibility of their production in undemanding expression systems such as *E. coli* or *P. pastoris* makes fungal hyaluronidases interesting for industrial application. Reaction kinetics of mammalian hyaluronidases is complicated due to two-step reaction mechanism, multiple potential cleavage sites in a single substrate molecule, enzyme affinity dependent on substrate length and presence of transglycosylation. Occurrence of transglycosylation and relation between the substrate length and enzyme affinity was not investigated before in fungal hyaluronidases. Here we aim to shed some light on reaction kinetics of recombinant *Talaromyces stipitatus* hyaluronidase (TsHr) on short hyaluronan substrates.

We investigated reaction kinetics of TsHr using hyaluronan substrates 3 to 13 monosaccharides long using LC-MS. Tetrasaccharide with GlcA on non-reducing end was found to be the shortest substrate. Similarly to mammalian hyaluronidases, enzyme affinity increases greatly with the substrate length. We confirmed ability of TsHr to catalyze transglycosylation reaction as well. Even though amounts of products longer than substrates were almost negligible in many cases, we suggest that transglycosylation mechanism plays important role in substrate degradation by TsHr. This statement is based on ratio of reaction products found in particular reaction setups. Interestingly, connection between apparent V_{max} and the substrate length was not straightforward and the substrate ability to participate in transglycosylation reaction seems to have major influence on this parameter. Kinetics analysis of hyaluronan substrates differing in lengths and in flanking sugar moieties allowed for estimation of number and relative position of sugars that interact with active center prior to reaction.

Our research brought new information about fungal hyaluronidases, which are vital for their utilization in hyaluronan oligosaccharide preparation. Moreover, our data indicate that proportion of hydrolysis and transglycosylation mechanism on substrate degradation evaluated exclusively by “longer products” quantification might strongly underestimate the transglycosylation. Further research is needed to confirm this observation on other glycosaminoglycan hydrolases.

O12

Early type 2 diabetes screening by combining high-resolution capillary electrophoresis separation and machine learning supported data evaluation

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Summary

Diagnosis of type 2 diabetes in its early stages is currently a major challenge, emphasizing the need for rapid, simple, and reliable methods for the screening of the disease [1]. Our research aimed to develop a novel capillary electrophoresis-based analytical method suitable for the identification of potential N-glycan biomarkers from human blood before the disease appears and causes comorbidity. To obtain comprehensive profile information, the samples were treated with organic reagent (protein precipitation), enzymatically digested, fluorophore labelled and analysed by capillary electrophoresis [2][3]. Our results revealed detectable differences between the N-glycan profiles of close relatives who were healthy but later one became diabetic, and the other did not. The method may allow the prediction of diabetes before the onset of the disease, creating a new early screening method that can be easily integrated into routine diagnostics. In addition, clinical information from specific glycoprotein N-glycosylation profiles in diabetes may help to shed light on the underlying inflammatory pathophysiological processes and lead to new drug targets.

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O13

Analysis of brain tissue N-glycosylation using capillary electrophoresis

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Summary

Nowadays, diagnostic methods capable of early detection as well as treatments of various diseases are gaining increasing importance. This requires biochemical markers that can accurately identify different diseases and their various clinical stages. Since the 1960s, glycans have been playing a significant role as biomarkers, particularly for conditions such as cancer, inflammatory processes, and neurological diseases [1, 2, 3, 4]. Neurodegenerative disorders pose a substantial financial burden on healthcare systems worldwide due to the long-term treatment and rehabilitation costs. According to data from the World Health Organization, approximately 55 million people suffer from dementia globally [5]. The progressive nature of this disease suggests that its prevalence is expected to increase in parallel with an aging population. Alzheimer's disease, which accounts for 60–70% of all dementia cases, is one of its most common forms [6]. In my work, the glycan profiles of brain tissue samples were analyzed using capillary electrophoresis coupled with laser-induced fluorescence detection (CE-LIF). This approach provided valuable insights into the sugar structures specific to various regions of the brain tissue, enabling high-resolution "glycan mapping". In the initial phase of my research, the N-glycan profile of porcine brain tissue was examined, optimizing sample preparation parameters, with a focus on implementing the defatting steps and ensuring the reproducibility and robustness of the sample preparation protocol. The further aim of my work was to process human brain tissues using the optimized method and create specific glycomic maps associated with various neurological diseases, with particular emphasis on Alzheimer's disease. Additionally, we aim to establish a clinically relevant database to provide glycomic insights into the molecular level in the human brain. This database could enable the identification of new biomarkers, contributing to the early diagnosis of neurological diseases, such as Alzheimer's disease, and potentially facilitating the development of novel therapeutic approaches.

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O14

Data-driven gradient optimization in LC-MS/MS: From semi-experimental framework to applications in food phenolic analysis

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Summary

Designing suitable gradient programs in LC-MS/MS continues to be a demanding task, especially for targeted applications where chromatographic resolution has to be balanced against the operational constraints of tandem mass spectrometry. This work presents a conceptual framework that unifies chromatographic and mass spectrometric considerations into a single strategy for gradient design. The approach is built on three principal parameters: chromatographic dispersion (expressed as interquartile range, IQR), overlap of MRM acquisition windows (WO), and the overall method runtime (GT). These variables are mathematically integrated into a single evaluation index, the Gradient Score (GS), which enables objective assessment of different gradients [1].

Since the Gradient Score is ultimately intended as a tool for optimizing methods that involve a large number of analytes, predictive strategies were incorporated to expand beyond the experimentally tested compounds. Specifically, QSRR and LSS approaches [2] were employed to estimate retention times of phenolic compounds under various linear gradient conditions. This procedure yielded a comprehensive semi-experimental dataset, which was then used to select suitable gradient profiles for more than 200 phenolic analytes.

Beyond semi-experimental evaluation, the Gradient Score was also applied to guide method development for monitoring changes in the phenolic composition of apple vinegars during maturation and in coffee beans throughout roasting. Looking forward, we envision the Gradient Score as a versatile optimization tool, not only for gradient design but also for systematic comparisons of different columns, mobile phases, and related conditions.

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O15

Fully automated sample preparation in-syringe online coupled to HPLC for vitamins analysis in vegan milk

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Summary

Among the typical sample preparation methodologies applied for the analysis of organic components in milk are conventional solvent-assisted protein precipitation, centrifugation, separation of the supernatant, solvent evaporation, and reconstitution. While effective, this workflow is labor-intensive and difficult to automate. Fully automated approaches, such as flow-batch techniques, Lab-in-Syringe systems [1], have been applied to protein precipitation [2], despite the potential to reduce time, minimize reagent consumption, and improve reproducibility. In this work, we developed a double Lab-In-Syringe (LIS) system and consequently automated method coupled online to HPLC for the determination of seven water-soluble vitamins—B1 (thiamine), B2 (riboflavin), B3 (niacinamide), B3 (nicotinic acid), B6 (pyridoxine), B9 (folic acid), and B12 (cyanocobalamin) in vegan milk alternatives. Salt-assisted homogeneous liquid–liquid extraction (SA-HLLE), implemented using an automatic syringe pump, was used to simultaneously denaturate proteins and separate the precipitated matrix components from oat, soy, hazelnut, almond, and coconut milks with fat contents ranging from 0.8 to 3.5 %. The organic extract was then transferred into a second syringe pump, where solvent evaporation was achieved. This approach allowed solvent evaporation and sample preparation to run in parallel with chromatographic separation, thereby increasing throughput to 6 samples per hour. The fully automated method demonstrated high reproducibility and repeatability (RSD < 6.3 %) and effective analyte recovery ranging from 69.4 to 111.9 % for the selected milk samples.

To our knowledge, this is the first report of automated matrix precipitation and solvent evaporation in a flow system. The achieved performance highlights the feasibility of fully automating sample preparation for complex liquid matrices, combining high reproducibility with efficient parallel operation of extraction, evaporation, and analyte separation.

This study was supported by Charles University SVV 260 782, GAUK project no. 206623, and the bilateral Mobility- & Capacity Building-Projekt Výzva 8J24AT supported by the Czech Ministry of Education, Youth, and Sports. This study was co-funded by the European Union under the ATEBIO project (Advanced Techniques for Biomedical Diagnostics, Project ID CZ.02.01.01/00/23_020/0008535).

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O16

Automated analysis of proteins in dried plasma spot samples

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Summary

Quantitative dried plasma spot sampling (qDPS) is an innovative and effective way to the collection of blood plasma samples [1,2]. A small finger prick provides a microvolume of blood that is applied to a separation device enabling purification of plasma from cells and cell residues. Resulting liquid is collected on collection disc allowing its effective recovery and subsequent analysis. This way of sampling can be performed at home, it is minimally invasive, requires only minuscule amounts of blood and simplifies the transport and handling of the sample [3]. Electrophoresis of proteins in blood serum and plasma is a commonly applied clinical test segregating proteins primarily into six fractions, including albumin and five types of globulins. The representation of the proteins in the sample can be related to pathological states of the organism, namely inflammation, liver and kidney diseases, multiple myeloma, and others [4]. This work is focused on the development of a fast, simple, and innovative capillary electrophoretic (CE) method for the determination of proteins in plasma collected by qDPS. The elution of proteins was carried out by deionized water directly in a CE vial containing the qDPS and was optimized for fast and efficient sample processing. Conditions for the CE separation of proteins and interferents from the blood matrix and the qDPS sorbent were also optimized. In the future, the method will be fully automated, and all operations will be facilitated by a single commercial CE instrument, including the eluent transfer to the qDPS, elution of proteins from the collection disk, eluate homogenization, and subsequent analysis. Method validation and comparison with standard plasma and serum samples will be performed.

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O17

Nanofiber-based microextraction for environmental analysis

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Summary

Environmental contamination by trace-level pollutants poses a growing risk to ecosystems and human health, necessitating highly sensitive, selective, and sustainable analytical methods. Traditional sample preparation techniques often fall short in terms of efficiency, environmental friendliness, and adaptability to modern analytical requirements. Polymer-based nanofibers have become a hot trend in sorbent development due to their hydrophobic nature and high affinity for lipophilic analytes. We evaluated nanofibers made from biodegradable and synthetic polymers as sorbents in a novel spin-filter fiber-microextraction format. Biodegradable polycaprolactone (PCL) fibers produced via meltblowing exhibited strong hydrophobic interactions and a highly porous structure, ensuring efficient extraction of diverse analytes from river water. The spin-filter format enabled activation, extraction, and elution in just 10 s per step, completing a full extraction in 30 s, and allowed simultaneous processing of up to 48 samples with minimal manual handling. Published study demonstrated high recoveries of various xenobiotics (81.8–105.2%) and excellent repeatability (RSD 1.1–3.6%), with cartridges reusable for multiple extractions [1]. Building on this, we applied nanofiber spin-filter extraction to polycyclic aromatic hydrocarbons (PAHs), achieving similarly high recoveries and rapid processing times, confirming the versatility of the approach. Among the tested materials, biodegradable PCL showed strong retention of all PAHs through hydrophobic interactions, while aromatic polyphenylene sulfide (PPS) performed best for higher-ring PAHs due to combined hydrophobic and π–π interactions. The high efficiency of these nanofibers is attributed to their morphological properties such as porosity, surface area, pore size, and disc thickness as well as the type of polymer and its specific interactions with target analytes. Overall, these results demonstrate that nanofiber sorbents in spin filter devices offer a fast, efficient, and versatile alternative for microextraction of diverse contaminants from environmental waters.

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O18

An automated platform for the monitoring of microfluidic immobilized enzyme reactors

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Summary

The efficient use of biocatalysts, such as enzymes, is essential for achieving the goal of integrating eco-friendly chemistry into industrial processes. The use of purified enzymes can facilitate highly selective reactions under milder and greener conditions. In addition, immobilization of these enzymes simplifies it's removal from the reaction bulk, and allows for incorporation into continuous-mode, flow reactors. However, the preparation costs of the enzyme are still significant, and due to their sensitive biological nature, typically require optimization before implementation. Therefore, an optimization process requiring minimal amounts of enzyme is desired. Miniaturization, in the form of microfluidic immobilized enzyme reactors (MIERs) provides a partial solution to this issue. [1]

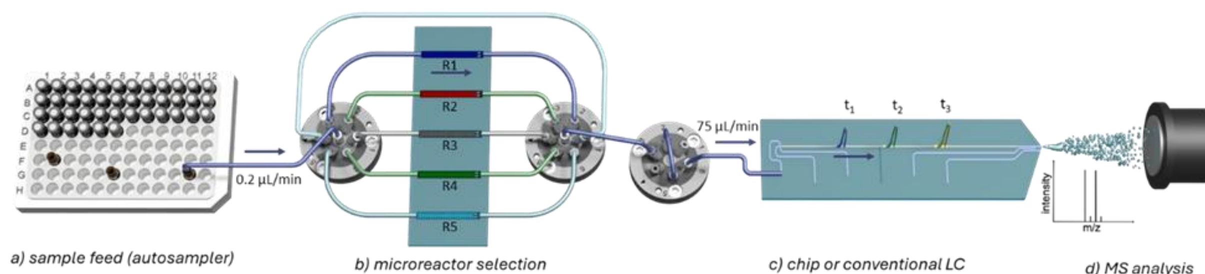

Figure 1. Simplified schematic of the automated multi-microreactor setup with on-line (chip)-LC/MS-detection [1].

We present a novel microfluidic optimization setup with real-time analytics for investigating enzyme catalyzed reactions utilizing multiple packed-bed MIERs. The setup combines microreactor technology, multi-reactor integration, and online LC/MS analysis in a sequential automated workflow, allowing for the generation of high quality data sets on the performance of the immobilized enzyme in continuous flow. The information obtained has the potential to be further used to upscale or number up the continuous process. In addition, a faster, modular chipLC solution was also tested as an alternative to the conventional LC, reducing overall solvent consumption by over 80%. Hence, providing an efficient platform for optimizing biocatalytic reactions with both minimal enzyme and reactant use. [1]

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O19

Novel multipurpose matrix for a high resolution MALDI MS and multimodal imaging

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Summary

The importance of multimodal imaging, combining two or more techniques for mapping tissues, is growing across medicine, chemistry, and biology research.[1] Multimodal matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) from a single tissue section is often hindered by limited inter-modality compatibility and complex matrix application procedures.[2] In this contribution, we present a simplified and reproducible high spatial resolution multimodal MALDI MSI combined with fluorescence and histological annotation, all allowed by a tissue staining with dye-based matrix – basic fuchsin. The staining protocol is inspired by our latest work employing staining with Basic Blue 7 matrix for MALDI MSI.[3] The staining with basic fuchsin streamlines sample preparation, eliminating the need for matrix spraying or sublimation, and enables true multimodal analysis on a single tissue section thanks to its compatibility with all employed imaging modalities. Innate fluorescence of basic fuchsin ensures that no additional sample treatment or addition of other fluorophores is needed for recording fluorescence images. The technique is demonstrated on mapping of mouse brain: MALDI MSI modality visualizes spatial distribution of lipids and fluorescence combined with histology clearly defines the different areas and tissue types in the brain section. Furthermore, MALDI MS with basic fuchsin is usable with laser postionization, i.e. MALDI-2, to increase sensitivity and spatial resolution to 5 micrometers. The high-quality of recorded images is comparable to the MALDI/MALDI-2 MSI with classical matrices. The developed multimodal workflow facilitates high-resolution molecular imaging and offers tools for holistic characterization of biological tissues.

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O20

Impedance-based detection of biofilm formation of *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Summary

Bacterial biofilms are complex microbial communities that adhere to surfaces and contribute to the pathogenesis of chronic infections. Therefore, it is crucial to detect biofilm-associated infections as their treatment becomes more complicated. Herein, we describe a label-free electrochemical impedance spectroscopy (EIS) method for detecting biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Printed circuit board-based biamperometric gold electrodes were modified with poly-L-lysine to enhance bacterial attachment to the sensor surface [1]. Formation and inhibition of formation of biofilms were evaluated based on changes in charge transfer resistance (R_{ct}). The control R_{ct} value increased by ~90 kΩ for *S. epidermidis* biofilm and by ~60 kΩ for *S. aureus* biofilms [2]. The antibiotic-treated samples exhibited values similar to the control. Furthermore, biofilm formation on electrodes was confirmed through optical microscopy, and atomic force microscopy (AFM) was used to visualize the biofilm on the electrode surface and evaluate its roughness. The roughness parameters indicate that *S. aureus* forms a rougher biofilm than *S. epidermidis,* while *S. epidermidis* produces more compact biofilm and a higher amount of extracellular polymeric matrix. These findings suggest that the optimized EIS-based method effectively monitors changes related to biofilms and serves as a promising tool for evaluation of new anti-biofilm agents, such as antibiotics, phages or antibodies.

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O21

Bioconjugates of upconversion nanoparticles for immunochemical detection of tumor biomarkers

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Summary

Sensitive detection of clinical biomarkers is crucial for the diagnosis of numerous illnesses. High sensitivity is of the utmost importance especially in the case of tumor diseases, allowing for early-stage cancer diagnosis and monitoring of treatment response. Due to their high specificity conferred by antibodies, immunochemical assays have proven indispensable for biomarker detection. However, conventional immunoassay labels, such as enzymes or fluorophores, often provide insufficient sensitivity for the detection of low-abundance biomarkers. Therefore, various kinds of nanoparticles have been investigated as labels to enhance immunoassay performance. Photon-upconversion nanoparticles (UCNPs) stand out as one of the most promising options. These lanthanide-doped nanocrystals possess the ability to convert near-infrared radiation into light of a shorter wavelength (anti-Stokes emission), significantly reducing the optical background interference. Moreover, their emission spectra can be easily tuned by altering the composition of dopant ions.

In the field of immunochemical methods, the heterogeneous assay format is predominant due to its high specificity and sensitivity. However, these desirable properties are ensured by immobilization and washing steps, leading to a prolonged procedure. In contrast, homogeneous immunoassays omit these time-consuming processes, however, at the cost of reduced specificity and sensitivity.

To combine the advantages of both the assay formats, we have developed a novel artificial intelligence-aided homogeneous immunoassay technique based on massively parallel spectroscopy (MPS) [1]. This single-molecule method utilizes two different UCNP-antibody labels with distinct emission spectra binding to the analyte molecule, detecting only sandwich immunocomplexes containing the analyte molecule and both labels with distinct emission spectra. The whole mixture is observed by modified upconversion microscopy, where the sandwich immunocomplexes appear as double spots, whose number is assessed by a neural network. We have successfully employed MPS in assays for prostate-specific antigen and protein p53, two important cancer biomarkers. Such immunoassay format was utilized for the first time, showing a strong potential to become a fast and high-throughput bioanalytical method [2].

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O22

Physics for sensitivity, chemistry for selectivity: Planar spark and Ag/Fe_xO_y colloid substrates for SERS

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Summary

Surface-enhanced Raman spectroscopy (SERS) is an ultrasensitive analytical technique whose broader analytical use remains limited by the reproducibility and functional selectivity of available substrates.

We address these problems with two strategies: Planar SERS fabricated by 3D-positioned spark discharges forms dense Ag nanostructures directly on conductive wafers and can operate in a clean dielectric environment (air). Performance was optimized by high-throughput Raman mapping: limit of detection for adenine is in picomolar range [1].

Affinity-driven SERS with Ag/Fe_xO_y nanocomposites selectively captures phosphorylated analytes on iron oxides while Ag provides the plasmonic readout. Affinity can be further promoted at acidic pH, enabling capture-and-probe of phosphate-bearing analytes (e.g., AMP) in complex matrices [2].

These results outline a practical choice: maximize Raman scattering enhancement for ultralow-level detection on planar substrates or use chemical affinity to pre-select targets in complex matrices.

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O23

Development of microfluidic capillary electrophoresis with surface-enhanced Raman scattering detection (CE–SERS)

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Summary

Raman spectroscopy is a powerful analytical technique with a wide range of potential applications. However, its wider use is mainly limited by its low sensitivity. This disadvantage can overcome surface-enhanced Raman spectroscopy (SERS), a derived method that uses metal nanostructures in close proximity to the analyzed substance to amplify the Raman signal. Another challenge of Raman spectroscopy is the complexity of obtained spectra. While this is extremely beneficial for detailed characterization of a single analyte, it challenges the interpretation of individual signals coming from mixtures. To overcome these limitations, we are working to develop a system that combines the separation efficiency of capillary electrophoresis (CE) with the high-sensitivity SERS detection.

A promising platform for the integration of CE and SERS is a microfluidic chip. Microfluidic devices offer numerous advantages, as they enable miniaturized and controlled environments for chemical reaction, separation and detection. [1] In this work, we present the fabrication of borosilicate glass microfluidic chips using photolithography and wet etching. The separation conditions for chip-CE were optimized through fluorescence-based detection of selected analytes. Special attention was paid to voltage conditions (Figure 1A), composition of BGE, and loaded volume.

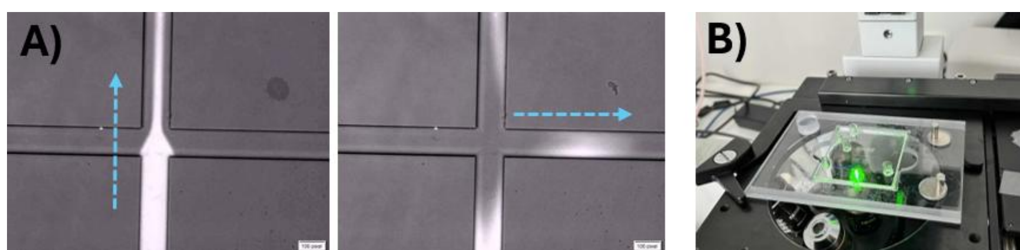

Figure 1 A) Introduction of the analyte into the separation channel of the microfluidic chip by a change of voltage conditions. The arrow shows the direction of the analyte's flow. B) Photograph of the glass chip used in this work.

This study further proposes a chip design with an additional side channel that would be adapted for future implementation and acoustic focusing of metal nanoparticles (Figure 1B). This modification is a key step towards

integrating SERS detection into CE flow without a loss of separation efficiency and could eventually form a versatile analytical tool.

This work was supported by the Czech Science Foundation for financial support (Grant No. 25-16617S). Additional support was provided by a grant from the Programme Johannes Amos Comenius under the Ministry of Education, Youth and Sports of the Czech Republic (project ID CZ.02.01.01/00/23_020/0008535 co-financed by the European Union), Mobility Plus Project (VAST-24-02) awarded by Czech Academy of Sciences, and by institutional support RVO 68081715 of the Institute of Analytical Chemistry of CAS (Czech Republic).

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O24

Deuterated water amplified fluorescence detection in capillary electrophoretic separation of DNA

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Summary

Separation of DNA fragments by capillary electrophoresis is a widely used commercial method. It is performed in a gel sieving medium in combination with fluorescence detection using DNA-intercalating dyes. The fluorescence intensity can be influenced by many factors; one of these groups is the physicochemical propertiesof the solvent (background electrolyte in the case of electrophoresis). Compared to ordinary water, deuterated water exhibits lower fluorescence quenching and reduced absorbance of excitation radiation, which often results in an increased fluorescence signal [1].

Deuterated water also affects capillary electrophoresis separation by decreasing the electroosmotic flow, thereby improving the resolution of separations and prolong the analysis time[2]. This work aims to investigate the influence of D_2O on the fluorescence of fluorescently labeled DNA and its electrophoretic behavior in capillaries.

Selected DNA stains were characterized using a spectrofluorometer, and the fluorescence intensities of DNA–dye complexes in normal and deuterated water were comparedto identify dyes most affected by solvent exchange for further electrophoretic experiments . The highest increase of fluorescence intensity were observed for ethidium bromide (2.8 times), propidium iodide (2.5 times) and Gel Green (2.0 times). The Agilent 5200 Fragment Analyzer (equipped with a blue excitation source) was used for DNA fragments separations. The background electrolyte containing Poly(N,N-dimethylacrylamide) gel was prepared in both H_2O and D_2O. DNA ladders labeled with the selected fluorescent dyes were used to evaluate the influence of D2O on capillary electrophoretic separation.

We confirmed that using D_2O influenced both migration time and fluorescence signal intensity. The combination of electrophoresis in D_2O-based media with dyes benefiting from reduced non-radiative relaxation provides a promising approach to enhancing the detection limits of DNA by capillary electrophoresis.

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O25

A decade of capillary electrophoresis–mass spectrometry in precision medicine: A global bibliometric study (2014-2024)

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Summary

Over the past decade, precision medicine has emerged as a transformative approach that tailors prevention, diagnosis, and treatment to individual genetic, molecular, and environmental characteristics [1]. This paradigm shift has been driven by the rapid expansion of omics sciences and by the need to overcome the variability of therapeutic efficacy in conventional medicine [2]. In this context, capillary electrophoresis coupled to mass spectrometry (CE-MS) has become a powerful analytical platform for molecular characterization and biomarker identification, contributing significantly to the advancement of omics-based and clinical research [3]. However, despite its growing importance, there is still a lack of systematic and quantitative assessments of how CE-MS has evolved and been applied within precision medicine. This study aimed to provide a comprehensive overview of global scientific production on CE-MS in precision medicine from 2014 to 2024. Data was collected from the Web of Science Core Collection and the Bibliometric and computational analyses were performed in Python. The results showed a continuous increase in publications over the decade, reflecting the consolidation of CE-MS as a relevant analytical tool in biomedical research. China leads both in the number of publications and citations, while the United States maintains a strong presence through institutions such as Harvard University. The most represented areas of research include Oncology, Pharmacogenomics, and Omics Sciences, indicating a clear clinical focus of CE-MS applications. The growing presence of artificial intelligence and data-driven approaches, demonstrates the field's technological evolution and methodological sophistication. Despite this progress, the analysis also highlights a strong geographical concentration of research output in high-income countries, with limited representation from Latin America and other emerging regions, indicating persistent disparities in access to advanced instrumentation and infrastructure. Overall, this bibliometric analysis portrays a decade of continuous growth, technological innovation, and increasing interdisciplinarity in CE-MS applied to precision medicine. The findings underscore both the maturity and the inequality of the field, emphasizing the importance of strengthening international collaboration and expanding participation of emerging research centers to foster a more inclusive and globally integrated scientific landscape.

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P1

Absolute quantification of nanoparticles and nanoplastics for their chemical characterization

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Summary

Nanomaterials possess unique physical and chemical features that drive their widespread use in various fields. However, their environmental persistence and interactions with living systems highlight the need for precise surface chemistry characterization. In this work, we developed an optical microscopy-based counting approach using anisotropically collapsing gels to quantify nanoparticles and their surface carboxyl groups. Advantageously, it allows for the characterization of new materials where calibration standards can be unavailable. The method was tested with poly(acrylic acid)-coated photon-upconversion nanoparticles (UCNP-PAAs), representing intrinsically luminescent probes for bioapplications, and carboxylated polystyrene nanoparticles (PNs), a model of environmentally relevant nanoplastics. The analysis revealed an average of 152 ± 14 thousand carboxyl groups per UCNP-PAA and 38 ± 3.6 thousand groups per PN, corresponding to ~11 and ~1.7 groups/nm^2, respectively. The limit of detection was 6.4 and 1.9 thousand carboxyl groups per nanoparticle, and the limit of quantification was determined at 21 and 6.2 thousand carboxyl groups per nanoparticle for UCNP-PAAs and PNs, respectively. Epifluorescence microscopy enabled direct imaging and counting of UCNPs, while PNs required additional staining with Nile Red. Acid-base titration with a colorimetric indicator was then used for quantification of carboxyl groups. This combined approach facilitates the accurate characterization of nanoparticle surface chemistry, providing structural insights that extend beyond the capabilities of electron microscopy. The study presents a robust strategy to investigate the influence of surface chemistry on nanoparticle functionality, design, and environmental behavior, with potential applications across a wide range of nanomaterials.

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P2

Fluorescein-based markers of isoelectric point as a tool tracking of pH gradient in highly sensitive isoelectric focusing analysis with laser-induced fluorescence detection

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Summary

The capillary isoelectric focusing (cIEF) instrumentation can be coupled with on-line laser-induced fluorescence (LIF) detection, which significantly increases detection sensitivity. However, there was considerable lack of any suitable p*I* markers for this type of application. In 2002 our laboratory devised fluorescein-based low-molecular-mass p*I* markers initially covering narrow pH range (from 5.4 to 6.6). Since then, by adding various functional groups, more than 60 further candidate ampholytes covering broad range of pH have been synthetized. However, some of the nonconforming compounds exhibiting low fluorescence intensity were excluded from the set. Utilizing the cIEF-LIF method the new markers were calibrated and total of 21 most suitable markers were characterized. Finally, based on the thorough analysis, four calibrated p*I* markers, evenly covering the whole length of the pH gradient, were selected and further applied for cIEF-LIF analysis and p*I* determination of fluorescently labeled antibody (IgG-FITC) and cell lysate from *Spirulina* sp., containing phycobiliproteins with naturally occurring fluorescence.

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P3

Characterization of linear and branched cuticular hydrocarbons in *Blaptica dubia* using GC/Q-TOF and molecular sieve separation

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Summary

Cuticular hydrocarbons (CHCs) play essential roles in insect physiology and communication. CHCs typically consist of complex mixtures of linear alkanes and alkenes, and methyl-branched hydrocarbons [1]. In this study, CHCs were extracted from the cockroach *Blaptica dubia* using chloroform and purified via two thin-layer chromatography steps to isolate saturated hydrocarbons. Molecular sieve zeolite 5A enabled selective and complete removal of linear hydrocarbons from the sample; 50 mg of molecular sieve was sufficient to treat 300 µg of the saturated hydrocarbon mixture. Qualitative analysis before and after molecular sieve separation was conducted using gas chromatography coupled with quadrupole time-of-flight mass spectrometry (GC/Q-TOF), providing high-resolution analysis. This approach allowed novel identification of seven linear and fourteen branched CHCs in *Blaptica dubia*, including nonacosane, 3-methylnonacosane, triacontane, 3-methyltriacontane, hentriacontane, 3-methylhentriacontane, tritriacontane, and various dimethyl hydrocarbons. These results are consistent with previously described CHC profiles of other cockroach species in the genera *Blattella*, *Periplaneta*, and *Blaberus*, where long-chain alkanes with more than 23 carbon atoms and their 3-methyl-branched isomers are typically abundant [2-4]. Characterization of the CHC profile of *Blaptica dubia* provides new insights into the chemical ecology of this species.

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P4

Chromatographic techniques for the analysis of historical pharmaceuticals of plant origin

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Summary

The analysis of historical pharmaceutical preparations, some dating back to the 18th century or even earlier, presents a unique opportunity to study the life of our ancestors and their traditional knowledge [1]. The popularity of pharmaceutical preparations made of plants can be illustrated by their frequency in rarely preserved Baroque pharmacies, as well as their eventual presence in current pharmacopoeias. The equipment of a peculiarly preserved pharmacy from Prague is now part of the collections of the National Museum, and chromatographic techniques have proven to be ideal tools for its analysis [2]. The aim is to verify the authenticity of historical pharmaceutical preparations and study the stability and potential degradation of biologically active compounds, which are often characteristic of medicinal plants. To this aim, it is beneficial to employ a chemotaxonomic approach, which means trying to identify some specific markers. However, plants are a very variable and complex material; thus, different types of extraction and separation techniques are often necessary for the analysis. The analyte and its properties define which analytical method must be used. A very powerful method for most analytes is liquid chromatography, although gas chromatography remains an irreplaceable method for volatile ones. The extraction is a crucial step in the analysis because it influences the number of compounds available for separation. For HPLC analysis, polar and less polar common organic solvents are frequently used, whereas SPME is typically associated with GC-MS analysis. Finally, to obtain the most suitable reference material, replicating period recipes is sometimes indispensable. The results of our analysis [3−5] pointed out the usefulness of chromatographic techniques connected with mass detection, which enabled us not only to verify the botanical authenticity of the samples but also to demonstrate the remarkable stability of some of their compounds.

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P5

Bringing liquid chromatography out of the lab: A compact UV-Vis detector for portable systems

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Summary

The development of portable liquid chromatography (LC) systems has gained considerable interest, as they allow sensitive and efficient analysis directly at the point of sampling. A key challenge is the miniaturization of optical detectors, since the flow cell design strongly affects sensitivity and signal quality. Building on earlier work carried out in our laboratory [1,2], we present a compact UV-Vis detector that integrates a miniature light source, a fused-silica capillary flow cell with a linear design, and a small spectrometer connected through optical fibers. Comparative testing with gradient separations demonstrated performance similar to conventional laboratory instruments, while requiring much smaller amounts of sample and solvent. The compact configuration confirms the suitability of this approach for portable LC systems using microcolumns.

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P6

Co-cultivation of *Hypericum*-borne endophytes unlocked anthraquinone biosynthesis

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Summary

Medicinal species of the genus *Hypericum* are known as prolific producers of secondary metabolites with diverse biological activities, including anticancer, anti-inflammatory, antimicrobial, and neuroprotective effects which can also be found in *Hypericum*-borne endophytic fungi. An important example is the production of naphthodianthrone hypericin in endophytes isolated from *Hypericum* spp. [1, 2]. Recent genomic analysis of putative anthraquinone biosynthetic gene clusters (BGCs) in these endophytes [3] underscores their remarkable metabolic potential. As many of these BGCs remain silent in axenic culture under standard laboratory conditions, co-culture has emerged as a promising approach to activate cryptic BGCs. The aim of this study was to assess the effects of different culture conditions on anthraquinone production in *Hypericum*-borne endofytes during co-culture. Six monocultures i.e., *Fusarium oxysporum*, *Plectosphaerella cucumerina*, *Scedosporium apiospermum*, *Canariomyces subthermophilus*, *Diaporthe eres* and *Septoria* sp., and their fifteen pairwise combinations were cultured on five different commercially available solid media. Based on morphological interactions between endophytes observed on solid media during co-culture, six combinations were selected for submerged static culture in liquid media. Medium and mycelium samples were collected in 7-day intervals over 35 days, extracted with ethyl acetate, evaporated to dryness, and redissolved in methanol. Metabolite profiles were analyzed using high-performance liquid chromatography (HPLC). Among monocultures, anthraquinone production was recorded only in *C. subthermophilus*. Besides emodin, frangulin B was also detected in potato dextrose broth (PDB), representing the first report of this metabolite in fungi. In the co-culture of *C. subthermophilus* and *Septoria* sp. chrysophanol was produced in addition to anthraquinones detected in monoculture. In PDB, chrysophanol was present in both the culture medium and mycelium, whereas in malt extract broth (MEB) it was restricted to the hyphae. These results indicate a stimulatory effect of *Septoria* sp. on chrysophanol production. Taken together, the findings demonstrate that fungal co-cultivation represents a promising strategy to activate otherwise silent biosynthetic pathways, thereby enhancing the production of bioactive metabolites with potential applications in drug discovery and biotechnology.

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Acoustic alignment of bacteria toward piezoelectric biopolymer devices

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Summary

Biodegradable functional materials are increasingly important for next-generation biosensors and bioelectronic devices. Among them, the piezoelectric biopolymer polyhydroxybutyrate (PHB), produced by the bacterium *Cupriavidus necator* H16, has gained particular attention. PHB exhibits piezoelectric properties when crystallized in a defined orientation. However, achieving such orientation in its native state is challenging due to the spherical morphology of intracellular PHB granules. This work focuses on developing more effective approaches for orienting this polymer. In this work, we present oriented structures of *C. necator* cultures obtained using surface acoustic waves (SAWs) and their subsequent analysis. After bacterial alignment, atomic force microscopy (AFM) images with 512 × 512 px resolution were acquired. Bacterial edges were detected, and those meeting user-defined conditions were fitted with a line. From these fits, the standard deviation from the mean orientation and the degree of orientation parameter (*R*) were calculated. The entire process was performed using in-house developed software designed specifically for this application. Representative AFM images and processed results are shown in Figures 1 and 2.

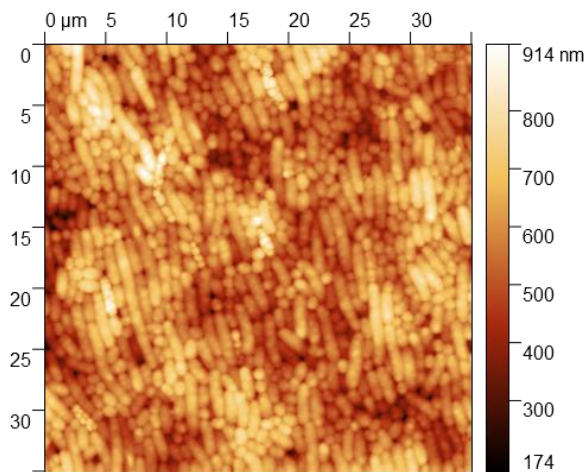

Figure 1: AFM image of oriented Cupravidius necator

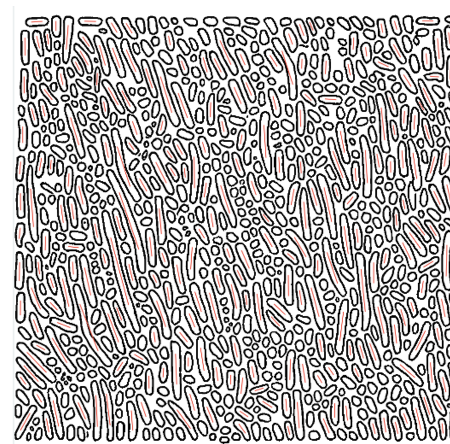

Figure2 : Highlighted edges of bacteria with SW line fitting

P8

Optimization of electrospray ionization for MS/MS sequencing of substance P

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Summary

Mass spectrometry (MS) is a highly sensitive analytical technique that allows detailed structural characterization of biomolecules from small metabolites to large and complex molecules. Electrospray (ESI) is one of the most important ionization methods. As it makes possible to transfer non-volatile molecules from the liquid phase at atmospheric pressure without significant fragmentation. For this reason, ESI-MS has become primary tool in proteomics.

Stability of the electrospray is very important factor directly impacting quality of MS signal. The efficiency of ionization process is affected especially by the emitter geometry, flow rate, applied voltage, and composition of the spray solution (Figure 1). Adding organic solvents to the sprayed sample reduces surface tension and increases volatility, while low concentrations of organic acids provide efficient ionization. In this work, we investigated the properties of so-called nanoelectrospray (nanoESI), which applies the principle of electrospray at very low flow rates. Emitter tips were fabricated from fused silica capillaries by symmetrical grinding using a specially designed device. The spray was generated either by attaching the emitter to an in-house built pressurized chamber or, alternatively, by integration with a microfluidic chip. The latter option enables more complex on-chip operations prior to spraying (e.g., online supply of additives or microfluidic separation).

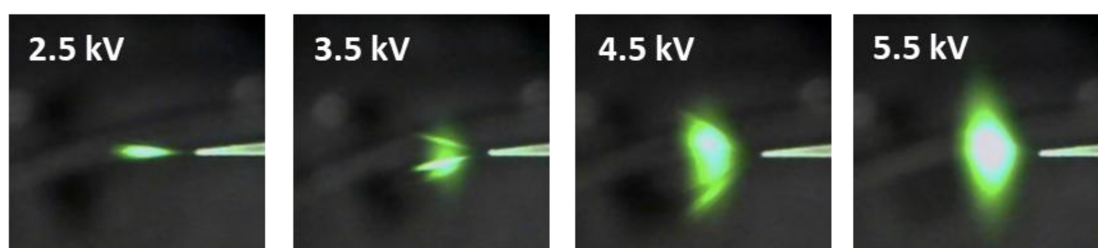

Figure 1: Appearance nanoESI plume at various voltage (values indicated in the figure). Experimental conditions: spray solution – 1% (v/v) HCOOH, flow rate – 140 nL/min, emitter inner diameter – 25 µm.

The optimized nanoESI parameters were used for MS/MS analysis of Substance P. Substance P, an 11-amino-acid neuropeptide, belongs to the tachykinin family, which acts as a neurotransmitter and neuromodulator in the central nervous system. Substance P plays an important role in transmitting signals related to pain, inflammation, and stress. Determination of its exact primary structure is essential for understanding its

biological functions. For this purpose, MS/MS sequencing was applied, enabling detailed peptide fragmentation and reconstruction of the amino acid sequence with high sequence coverage.

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